Waters

RECENT ADVANCES IN UPLC™ COLUMN CHEMISTRY

Eric S. Grumbach, Thomas E. Wheat and Jeffrey R. Mazzeo
Waters Corporation, 34 Maple St., Milford MA 01757

OVERVIEW

UPLC™ allows chromatographers to develop higher resolution, faster chromatographic methods by improving system efficiency. This is achieved by employing 1.7 µm particle packed columns and low dispersion, high pressure instrumentation.

It is current practice to improve sample throughput by employing shorter length columns at fast flow rates. Although this approach has merit to improve productivity, critical information is often lost when using conventional 3 and 5 µm particle packed columns. To improve the quality of information produced per unit time, short 30 mm 1.7 µm UPLC columns have been introduced to achieve rapid screening methods without compromising system efficiency.

Once initial method screening is conducted, it is often useful to further improve resolution by scaling the method to longer length UPLC columns to improve sample characterization. This exercise leads to informed decisions as to a desirable balance between analysis time and resolution.

Elevated temperature can also be used to improve resolution and analysis time. Temperature capabilities of the ACQUITY UPLC™ system have been extended to allow the use of longer length columns at faster flow rates to achieve higher resolution separations, rapidly.

In this poster, we discuss the efficacy of UPLC for improving sample throughput and resolution by manipulating column length and temperature.

EXPERIMENTAL APPROACH

A 30 mg/mL solution of doxylamine prepared in DMSO was used to show the usefulness of using an ACQUITY UPLC system for fast, high resolution impurity profiling.

A high throughput screening approach was conducted on a 30 mm UPLC column at acidic and alkaline pH to monitor the effect of analyte loadability related to the charge state of the analyte.

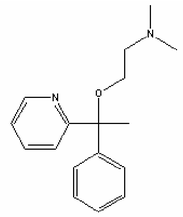
The method was geometrically scaled to longer length UPLC columns to improve the peak resolution and sample characterization.

The relationship between temperature and selectivity is discussed.

Elevated temperatures were then employed in combination with longer length UPLC columns to monitor changes in selectivity with temperature and improve sample characterization.

FAST METHOD SCREENING

The quality and safety of pharmaceutical drugs is impacted by the presence and quantity of process impurities. Therefore, it is important to screen a number of variables that manipulate selectivity in an efficient and timely manner to harvest useful data in a short period of time, and to make informed decisions that lead to a comprehensive characterization of the sample. A 30 mm UPLC column was used to quickly screen doxylamine and its impurities at acidic and alkaline pH.



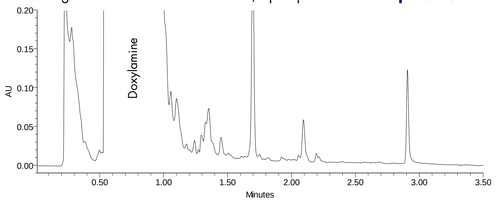
Doxylamine

Molecular weight: 279.38
pKa = 8.68 ± 0.28

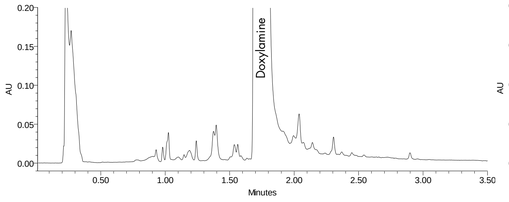
30 mg/mL in DMSO

The parent compound was intentionally overloaded to monitor the presence of low level impurities in the sample.

ACQUITY UPLC™ BEH C_{18} 2.1 x 30 mm, 1.7 µm 40 °C 0.4 mL/min
3 minute gradient 10—85% Acetonitrile,6 µL injection **pH 3.25**



ACQUITY UPLC™ BEH C_{18} 2.1 x 30 mm, 1.7 µm 40 °C 0.4 mL/min
3 minute gradient 10—85% Acetonitrile,6 µL injection **pH 9.25**

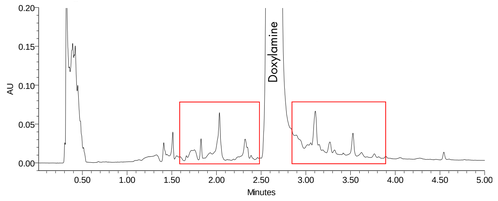


Manipulation of the mobile phase pH changes the ionization state of doxylamine which results in improved loading capacity and peak resolution of the parent compound and its impurities at alkaline pH. To improve characterization of the sample, the initial screening method can be scaled to longer length columns.

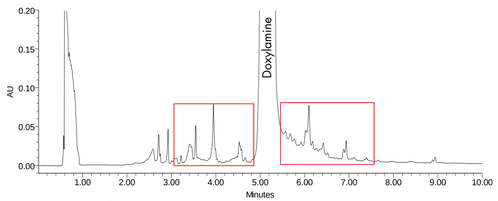
IMPROVING RESOLUTION

Once initial method screening is conducted on the 30 mm column, the method can be geometrically scaled to longer length columns to improve chromatographic resolution and sample characterization. The number of gradient column volumes and injection volumes, must be scaled appropriately to preserve the selectivity of the separation and peak profile, respectively.

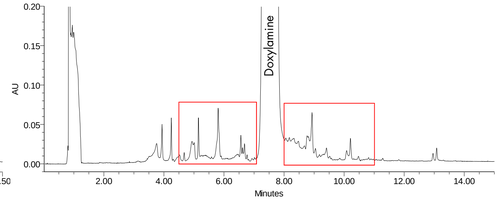
ACQUITY UPLC™ BEH C_{18} 2.1 x **50 mm**, 1.7 µm 40 °C 0.4 mL/min
5 minute gradient 10—85% Acetonitrile 10 µL injection pH 9.25



ACQUITY UPLC™ BEH C_{18} 2.1 x **100 mm**, 1.7 µm 40 °C 0.4 mL/min
10 minute gradient 10—85% Acetonitrile 20 µL injection pH 9.25



ACQUITY UPLC™ BEH C_{18} 2.1 x **150 mm**, 1.7 µm 40 °C 0.4 mL/min
15 minute gradient 10—85% Acetonitrile 30 µL injection pH 9.25

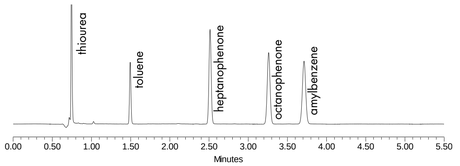


Improvements in chromatographic resolution can be observed with increasing column length. Combining elevated column temperature with higher resolution 150 mm UPLC columns may prove useful in exploring selectivity and shortening analysis time.

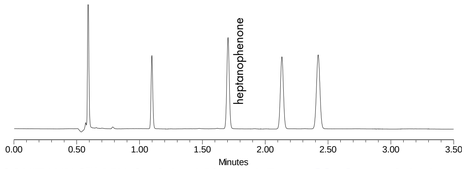
PRINCIPLES OF TEMPERATURE

Operating at elevated temperatures can provide several advantages including; improved analyte diffusivity, higher optimal flow rates therefore shortening analysis time and increased analyte solubility. Additionally, reduced solvent viscosity and system backpressures allow the use of longer length columns packed with smaller particles to be run at faster flow rates to produce high resolution separations in shorter analysis times.

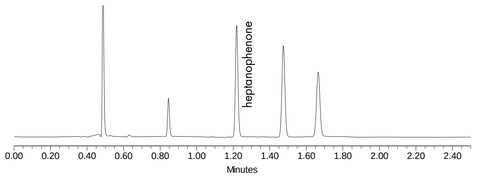
ACQUITY UPLC™ BEH C_{18} 2.1 x 150 mm, 1.7 µm **60 °C** 0.4 mL/min
70% Acetonitrile Run Time = 4 min **$N_{Heptanophenone}$ = 35,799**



ACQUITY UPLC™ BEH C_{18} 2.1 x 150 mm, 1.7 µm **75 °C** 0.5 mL/min
70% Acetonitrile Run Time = 2.5 min **$N_{Heptanophenone}$ = 35,643**



ACQUITY UPLC™ BEH C_{18} 2.1 x 150 mm, 1.7 µm **90 °C** 0.6 mL/min
70% Acetonitrile Run Time = 2.0 min **$N_{Heptanophenone}$ = 35,140**

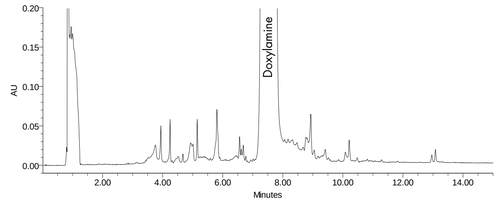


Increasing the column temperature from 60 to 90 °C results in a 50% decrease in analysis time while maintaining system efficiency. Although elevated temperature appears to be an attractive option for reducing analysis time, the relationship between temperature and selectivity is not linear.

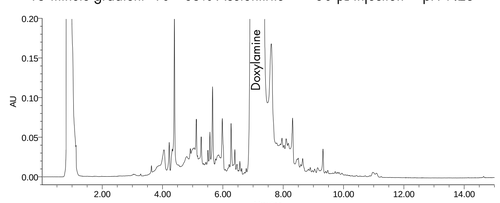
TEMPERATURE AND SELECTIVITY

It is important to recognize that temperature is a useful tool to manipulate selectivity. However, changing the column temperature may result in *unpredictable selectivity changes* that may necessitate further manipulations of the method.

ACQUITY UPLC™ BEH C_{18} 2.1 x 150 mm, 1.7 µm **40 °C** 0.4 mL/min
15 minute gradient 10—85% Acetonitrile 30 µL injection pH 9.25



ACQUITY UPLC™ BEH C_{18} 2.1 x 150 mm, 1.7 µm **90 °C** 0.4 mL/min
15 minute gradient 10—85% Acetonitrile 30 µL injection pH 9.25



By employing elevated temperatures, a major impurity of doxylamine becomes further resolved from the parent compound which previously co-eluted at lower temperatures.

CONCLUSIONS

- Short 30 mm 1.7 µm UPLC columns can be utilized to screen methods effectively and efficiently without compromising separation efficiency.
- Resolution can be improved by increasing column length which leads to informed decisions regarding the balance between analysis time and desired resolution.
- Elevated temperature allows the use of longer length higher resolution columns packed with 1.7 µm particles to be run at faster flow rates to produce high resolution separations in shorter analysis times.
- The relationship between temperature and selectivity is not linear and changing temperature may result in unpredictable changes in selectivity.
- Combining higher pressures, elevated temperatures and longer length 1.7 µm UPLC columns, provides the opportunity to extend the separation capabilities of UPLC.

TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS